REMARKS

The invention as presently claimed relates in part to assay methods for specifically detecting and/or quantifying BCR-ABL gene rearrangements. In particular, the presently claimed methods provide highly reproducible qualitative and quantitative results in which the presence and/or amount of three different BCR-ABL translocations may be determined in a single assay.

Certain lymphoblastic diseases develop when two genes, BCR on chromosome 22 and ABL on chromosome 9, recombine to form a hybrid BCR-ABL gene with leukaemogenic properties. The mechanism which underlies this recombination is unknown, but additional chromosome sites may be involved to form complex BCR-ABL rearrangements. In most chronic myeloid leukemias (CMLs), the BCR translocation region is known as the "major breakpoint cluster region" ("M-bcr"), and the resulting hybrid BCR-ABL genes typically comprise a "b2a2" or b3a2" junction, where the b2 or b3 region of BCR is joined to the a2 region of ABL. Similarly, in many acute lymphoblastic leukemias (ALLs), the BCR translocation region is known as the "minor breakpoint cluster region" ("m-bcr"), and the resulting hybrid BCR-ABL genes typically comprise an "e1a2" junction, where the e1 region of BCR is joined to the a2 region of ABL. Both CML and ALL are "clonal" diseases, meaning that single malignant progenitor cells proliferate into an abnormal population of cells, each containing an identical mutation. A schematic of the BCR and ABL genes, and the e1a2, b2a2 and b3a2 translocations, are as follows:

Analysis of these translocations may be performed using the "polymerase chain reaction," or "PCR," to amplify a nucleic acid of interest. PCR typically uses a pair of "primer" nucleic acids (referred to as a "forward" and a "reverse" primer) that flank the nucleic acid of interest, and which "prime" synthesis of a copy of the DNA between the probes when DNA polymerase is added. Following production of multiple copies of the target sequence, the copies are detected using labeled hybridization probes.

In the case of the present invention, a BCR e1 "forward" primer combines with an ABL a2 "reverse" primer to amplify a 219 base pair fragment if the e1a2 translocation is present; and a BCR b2/b3 forward primer combines with the same ABL a2 "reverse" primer to amplify a 124

base pair fragment if the b2a2 translocation is present, or a 199 base pair fragment if the b3a2 translocation is present. Detection of the amplification fragments is performed using two probes: a first specific for the e1a2 translocation, and a second specific for the b2a2/b3a2 translocations. In addition, a GAPDH gene is also amplified using a primer pair and a specific probe. See, e.g., specification, page 14 (describing the primers and probes), and page 28 (describing the fragments generated).

Thus, in the present invention, eight specific primers and probes (e1 forward, b2/b3 forward, a2 reverse, GAPDH forward, and GAPDH reverse primers; and e1a2, b2a2/b3a2, and GAPDH probes) set forth in SEQ ID Nos: 1-8 have been designed to differentiate between three BCR-ABL translocations, i.e., e1a2, b2a2 and b3a2, in a single assay, as well as to provide an internal GAPDH control fragment. The primers and probes are designed for such a "multiplexed" analysis, where the skilled artisan will understand care must be taken to avoid spurious "priming" of DNA synthesis by probe nucleic acids, primer/primer hybridization, primer/probe hybridization, etc. See, e.g., specification, page 15, first paragraph. The methods for distinguishing all three BCR-ABL translocation rearrangements in a single assay comprise reverse transcribing the RNA to cDNA, amplifying the cDNA, and detecting a cDNA signal. In preferred embodiments, the amplification and detection of the cDNAs are accomplished by "Real Time PCR," which refers to specific PCR methods in which a signal emitted from the assay is monitored during the reaction as an indicator of amplicon production during each PCR amplification cycle (i.e., in "real time").

As described in the specification on page 30, section 9.3.3, each BCR-ABL translocation provides an amplification product having a unique size. Figure 3 provides a visual demonstration of the ability of the present invention to detect each of these amplification products.

Claims 1-13 are presently pending in the instant application. Applicants respectfully request reconsideration of the claimed invention in view of the foregoing remarks.

Non Art-Related Remarks

35 U.S.C § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 2 and 3 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the present invention.

Applicable Legal Standard

When determining definiteness, the proper standard to be applied is "whether one skilled in the art would understand the bounds of the claim when read in the light of the specification." Credle v. Bond, 30 USPQ2d 1911, 1919 (Fed.Cir.1994). See also Miles Laboratories, Inc. v. Shandon, Inc., 27 USPQ2d 1123, 1127 (Fed.Cir.1993) ("If the claims read in the light of the specification reasonably apprise those skilled in the art of the scope of the invention, § 112 demands no more.").

The term "Real Time PCR" is well known and accepted by those of skill in the art

Applicants respectfully disagree with the Examiner's assertion that the phrase "real time PCR" in claims 2 and 3 is allegedly indefinite as "all PCR amplification reactions are conducted in real, as opposed to imaginary time." Paper No. 14, page 2. The phrase "real time PCR" is well known to those of skill in the art as referring to specific PCR methods in which a signal emitted from the assay is monitored during the reaction as an indicator of amplicon production during each PCR amplification cycle (i.e., in "real time"), as opposed to conventional PCR methods, in which an assay signal is detected at the endpoint of the PCR reaction. The description in Applicants' specification, as exemplified in the passage below is clearly consistent with the art understood meaning of "real time PCR."

> This assay is, in a preferred embodiment a real time quantitative RT-PCR assay using, for example, *ABI PRISM® 7700* Sequence Detection System, a thermal cycler associating a laser, a detector and **real time amplification detection software** (available from, for example, PE Biosystems). This technique

> allows the quantification of a specific target, using for example, a fluorescent labeled probe and the 5' nuclease activity of Taq DNA polymerase **during the PCR process.**

Page 3, lines 2-7 (emphasis added).

Applicants respectfully submit that, from the point of view of the skilled artisan, the phrase "real time PCR" is both well known and commonly used. As an indication of the acceptance of this phrase by those of skill in the art, Applicants determined from a search of the Medline database that there were 383 publications in which this term is used in the title. See, e.g., Applicants' response to the first office action. Indeed, the Examiner agrees that the phrase "real time PCR" is used in numerous publications by artisans, e.g., in 213 publications according to the Examiner's search. However, the Examiner argues that the publications do not use "real time PCR" to mean the same thing and cites to Dehée et al. and Aldea et al. as exemplary. Paper No. 14, page 11.

The Examiner is mistaken. Dehée et al. states that: "during each PCR cycle one molecule of reporter dye is cleaved for each target molecule amplified. The released reporter fluorescence is measured in real time." Dehée et al., page 39, right column, last paragraph. uses "real time PCR." Figure 2A of Dehée et al. which is a graph showing fluorescence results taken during each amplification cycle, also demonstrates Applicants' asserted meaning that "real time PCR" refers to monitoring amplicon production during each PCR amplification cycle. Similarly, Aldea et al. states that: "real-time PCR has started to demonstrate its potential utility.... The main features making this new technology so attractively suitable for these applications, in comparison to conventional PCR, are rapidness, possibility of accurate quantification, and... reduction of likelihood of contamination, since no postamplification analysis of the tubes is required." First paragraph (emphasis added). Figure 1 of Aldea et al., which is a graph showing fluorescence results taken during each amplification cycle, also support Applicants' asserted meaning that "real time PCR" refers to monitoring amplicon production during each PCR amplification cycle.

The Examiner's emphasis on the alleged differences in protocols and instruments between Dehée et al. and Aldea et al. is misplaced. It is of no moment whether Dehée et al. uses

the "Taqman" system or Aldea et al. uses a "lightCycler" instrument because "real time PCR" means the same in both publications as it does in the art as a whole -- the monitoring of amplicon production during each PCR amplification cycle as opposed to measurement of amplicon at the endpoint.[1] Thus, the fact that the authors of the publications cited by the Examiner use different protocols and instruments, but still recognize that each is using "real time PCR" to mean the same as asserted by Applicants and understood by the art as a whole, supports the conclusion that the skilled artisan is reasonably informed of the metes and bounds of the phrase "real time PCR." The Examiner's discussion of "imaginary time" as the alternative to "real time" evidences a failure to consider that interpretation of the claims is to be made in light of the knowledge of those of skill in the art.

As stated in MPEP §2173.02, "[d]efiniteness of claim language must be analyzed, not in a vacuum, but in light of... [t]he teachings of the prior art; and [t]he claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made." Applicants respectfully submit that, because the phrase "real time PCR" is commonly understood by those of ordinary skill in the art, and because Applicants have made clear that this commonly understood meaning applies to the phrase "real time PCR" as used in the instant claims, the skilled artisan is reasonably apprised of the scope of the present claims. 35 U.S.C. §112, second paragraph, demands no more. Moreover, if any latent ambiguity remains in the phrase, Applicants have clearly indicated that the meaning of the phrase "real time PCR" for purposes of the present application corresponds to its well established meaning in the relevant art.

In view of the above, Applicants respectfully request that the rejection be reconsidered and withdrawn.

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[1] For the convenience of the Examiner, a description of the LightCycler system by Roche Molecular Biochemicals is attached hereto. As noted on page 3, first paragraph, the lightcycler is a instrument designed for real time PCR ("[t]he LightCycler offers kinetic quantification, a fast, accurate way for quantification by PCR. Real-time, kinetic quantification allows measurements to be made during the log-linear phase of a PCR... [as opposed to methods in which] data were acquired only in the plateau phase of the PCR (end-point determination)").

Art-Related Remarks

35 U.S.C. § 103

Applicants respectfully request that the rejections of claims 1-6 and 8-13 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mensink *et al.*, *British J. Haematol.* 102: 768-774 (1998) in view of Hariharan *et al.*, *EMBO J.* 6: 115-119 (1978) and further in view of Shtivelman, *Cell* 47: 277-284 (1986), and of claims 1-13 under under 35 U.S.C. § 103(a) as allegedly being unpatentable over Eder *et al.*, *Leukemia* 3: 1383-89 (1999) in view of Hariharan *et al.* and Shtivelman, and in further view of Ercolani *et al.*, *J. Biol. Chem.* 263: 15335-15341 (1988) be withdrawn, as no *prima facie* obviousness has been established, or, in the alternative, any *prima facie* case of obviousness has been rebutted.

Applicable Legal Standard

To establish a prima facie case of obviousness, three criteria must be met: there must be some motivation or suggestion, either in the cited references or in knowledge available to the ordinarily skilled artisan, to modify or combine the references; there must be a reasonable expectation of success in combining the references; and the references must teach or suggest all of the claim limitations. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991) *See also*, MPEP §2143.

The Examiner has not met the initial burden required to combine the publications as suggested, thereby failing to establish a prima facie case of obviousness

As Applicants stated in the foregoing summary of the invention, the instant claims refer to methods employing a set of eight specific primers and probes. These nucleic acids have been selected to differentiate the presence of the e1a2, b2a2 and b3a2 BCR-ABL translocations in a single, multiplexed assay. In contrast, the primary reference, Mensink *et al.*, discloses a method for quantitation of a bcr-abl cDNA fragment using only a single set of primers and one probe for bcr-abl fragment. In addition, the primers disclosed in the Mensink *et al.* publication are different from those recited in the present claims. Similarly, the primary Eder *et al.* publication

discloses a method for quantitation of only the b2a2 and b3a2 variants of BCR-ABL, in this case using two pairs of primers and two probes. *See, e.g*, Eder *et al.*, abstract. Again, the Examiner does not assert that the primer pairs and probes disclosed in the Eder *et al.* publication are identical to any of the primers and probes of the present claims, much less that the Eder *et al.* publication discloses any assays capable of differentiating each of the e1a2, b2a2 and b3a2 BCR-ABL translocations.

Because the primary Mensink *et al.* and Eder *et al.* publications do not themselves disclose or suggest the instantly claimed invention, the Examiner seeks to combine each of the primary publications with the secondary Hariharan *et al.* and Shtivelman publications, which are cited as simply disclosing the complete DNA sequences of BCR and ABL, respectively. The Examiner contends that "the only significant difference between the prior art and the current claims is the particular primers selected from the BCR and from the ABL sequences" (Paper No. 14, page 12), and that the primers of the claims "simply represent structural homologs" of the primers in the Mensink *et al.* and Eder *et al.* publications (*id.*, page 6) which may be obtained from the complete BCR and ABL sequences. The Ercolani *et al.* publication is cited by the Examiner solely for the disclosure of a full length GAPDH sequence. *See, e.g.*, Paper No. 14, page 10.

Applicants respectfully submit that it is the Examiner's initial burden to establish a prima facie case of obviousness, and unsupported assertions that the primers of the claims "simply represent structural homologs" of the primers in the primary publications do not meet this burden. The Court of Appeals for the Federal Circuit has repeatedly cautioned that:

> [t]he factual inquiry whether to combine references of record must be thorough and searching. It must be based upon objective evidence of record.... [T]he best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.... [P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

In re Sang-Su Lee, 277 F.3d 1338, 1343 (2002) (internal citations omitted, emphasis added).

As Applicants previously noted, there is nothing supporting the obviousness rejection other than the bare assertion that the primers referred to in the instant claims are "structural homologues" of the primers disclosed in the primary publications. Applicants further note that, while the individual nucleotides making up typical nucleic acids are chosen from the same "alphabet" of A, T, G, and C, the relative arrangement of nucleotides provides unique structural and functional properties to any particular nucleic acid that are not "homologous" to other nucleic acids having a different arrangement of nucleotides. Applicants respectfully submit that nothing of record indicates a reasonable expectation that nucleic acids having different sequences will have similar properties; hence, no presumption of obviousness based on structural similarity is permitted. See, MPEP § 2144.09.

Applicants also take issue with the Examiner's conclusion that "[a]n ordinary practitioner would expect successful detection of the BCR-ABL translocation from every primer selected according to the methodology taught by Eder" (Paper No. 14, page 12). This conclusion is unsupported by any objective evidence and is, in fact, contrary to the understanding of those skilled in the art. Rather, the skilled artisan is well aware that each potential primer in a given nucleic acid sequence is not structurally or functionally equivalent. Taken to its logical conclusion, the Examiner's position renders any of the many thousands of potential primer sequences that might be obtained from a large nucleic acid molecule automatically prima facie obvious, without any need to provide evidence that any particular sequence will function as a primer at all.

In response to the first office action, Applicants explicitly requested that the Examiner provide some objective evidence in support of the assertions made in the rejection, so that the evidence might be considered and rebutted. In response, the Examiner offered only additional unsupported assertions. For example, the Examiner asserted that:

> "[t]he primers are all drawn from the identical sequence for the identical purpose, so an ordinary practitioner would have been motivated to select primers using software expressly disclosed by

> Mensink, the Primer Express software, where the software selects primers based on input criteria of desired Tm, length, and other well known parameters"

(Paper No. 17, page 2, first full paragraph). This assertion is not obtained from any prior art teaching, but merely springs from the Examiner's pen. The Examiner continues to ignore the need for particular findings, based on evidence of record, as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed. Moreover, the Examiner's assertion of "identical purpose" is clearly wrong, as the sequences of the present claims are selected to detect each of the e1a2, b2a2 and b3a2 BCR-ABL translocations together in a multiplex PCR (specification, page 15, first paragraph), while the sequences of the Mensink et al. and Eder et al. publications have not been so selected.

Applicants respectfully submit that, because the Examiner has failed to perform the thorough and searching factual inquiry necessary to support an assertion of obviousness, or based the rejection upon any objective evidence of record, the Examiner has failed to meet the burden of establishing a prima facie case of obviousness. Instead of analyzing the scope and content of the prior art, the level of skill in the art, the differences between the claimed invention and the prior art, and any objective indicia of non-obviousness, the Examiner has sought to short-circuit the inquiry by simply asserting "[a]n ordinary practitioner would expect successful detection of the BCR-ABL translocation from every primer selected," without objective support for such a statement. Paper No. 14, page 12.

The prior art demonstrates that the obviousness rejection is improperly founded and without basis

Applicants have filed the instant Request for Continued Examination in order to place into the record publications which rebut the unsupported premise upon which the rejection is founded, that any potential primer in a given nucleic acid sequence is structurally or functionally equivalent. In this regard, Applicants submit He *et al.*, *BioTechniques* 17: 82-87 (1994), which notes that primers that differ even "slightly" in position can exhibit 100- to 1000-fold differences in amplification sensitivity, and that "a trial-and-error" approach must be used to identify useful

primers. He *et al.*, abstract. Similarly, Applicants submit an excerpt from Robertson and Walsh-Weller, *Meth. Mol. Biol.* 98: 121-126 (1998), which confirms that, simply because a computer program is used to select sets of primers, the skilled artisan would not expect every primer set to be either structurally or functionally homologous. *See, e.g.*, Robertson and Walsh-Weller, pages 122-123 (while "there are guidelines, as reported by numerous authors, that may be useful in designing effective primers... [d]espite a gallant attempt at optimization of the PCR and primer design, poor sensitivity might only be relieved when new primer pairs are tried") (citing He *et al.*).

Furthermore, the Robertson and Walsh-Weller publication also cautions on page 124 that the results obtained from primer design software, such as that allegedly disclosed by the Eder *et al.* publication, must be "regard[ed]... with healthy skepticism." Current software algorithms suffer from the fact that "important factors which influence the stability of nucleic acids have yet to be identified." Kämpke *et al.*, Bioinformatics 17: 214-25 (2001), page 224, right column. This is particularly true in multiplex PCR assays, such as those of the instant claims. See, e.g., *id.*, page 214, right column ("The design complexity increases in so-called multiplex PCR.... [T]his requires that physical parameters such as cycle number, cycle duration and annealing temperature are identical for all of the PCR reactions. Moreover, the analysis of unintended primer-primer interactions becomes more intricate"). As a result, "a trial-and-error" approach continues to be required to obtain useful primers, particularly for multiplex primer sets.

When properly considered, it is apparent that the skilled artisan would clearly not expect successful detection of the BCR-ABL translocation from every primer selected from a particular sequence. It is equally apparent that the skilled artisan would not consider the primers recited in the instant claims, which together provide highly reproducible qualitative and quantitative results in which the presence and/or amount of three different BCR-ABL translocations, to be "structural homologues" of the primers disclosed in the cited publications. When the scope and content of the prior art is properly considered, it is clear that the Examiner's reasoning in support of the obviousness rejection is incorrect. Thus, Applicants respectfully submit that no *prima facie* case of obviousness has been established.

The superior properties of the claimed invention overcome any prima facie case of obviousness that may have been established

It is also respectfully submitted that the methods of the instant claims, which recite the use of eight specifically designed nucleic acid primers and probes, provide methods by which the presence and/or amount of three different BCR-ABL translocations may be distinguished in a single assay. As described in the specification on page 30, section 9.3.3, each of the e1a2, b2a2 and b3a2 BCR-ABL translocations for which the primers and probes of the present claims have been designed provides an amplification product having a unique size when amplified according to the claimed methods. Figure 3 provides a visual demonstration of the ability of the present invention to detect each of these amplification products.

In contrast, the cited Mensink *et al.* publication discloses one primer pair and one probe for detection only the b2a2 and b3a2 translocations, while the Eder *et al.* publication discloses two primer pairs and two probes for detection of these same translocations. *See, e.g.*, Mensink *et al.*, page page 773, left column, 23-25 ("The PCR primers were located in exon BCR2 and ABL exon 2. This enabled detection and quantitation of the most common B2A2 and B3A2 fusion transcripts"); Eder *et al.*, abstract.

Because none of the publications cited by the Examiner detects each of the e1a2, b2a2 and b3a2 BCR-ABL translocations in a single assay, the methods of the instant claims provide superior results as compared to the cited publications. Applicants assay is superior because it eliminates the need to run two separate assays as was required in the prior art. To the extent that a *prima facie* case of obviousness has been established, such a *prima facie* case is rebutted by this evidence of superior results. *See, e.g.*, MPEP §2144.09.

In response to Applicants' evidence in this regard, the Examiner asserts that "applicant's statement is not evidence and the specification lacks comparative data," and that these arguments are simply the "arguments of counsel." Paper No. 14, page 12. Applicants respectfully disagree with this characterization. The instant specification describes in detail the claimed methods "designed to be able to amplify and detect all three translocations of the BCR-ABL gene, namely e1a1, ba2a2, and b3a2, without interfering with each other and providing highly reproducible

results." Specification, page 15, first paragraph. The comparative data of record is provided by the specification, together with the fact that no publications of record provide any such assays. The Examiner must consider such data in the specification. *See, e.g.*, MPEP § 716.01(a).

The Examiner appears to content that a combination of the primers from the prior art references in a multiplex assay might be as useful at Applicants' method ("use of the cited prior art method itself in separate experiments might yield equally effective data" ; Paper No. 14, page 12). However, it is improper to require Applicants to compare the claimed invention with subject matter that does not exist in the prior art. *See, e.g.*, MPEP § 716.02(e) (citing *In re Geiger*, 815 F.2d 686, 689; 2 USPQ2d 1286, 1279 (Fed. Cir. 1987)). Applicants's invention need only be compared to the prior art single or double mutation assay, which is be definition inferior to a single assay of the same nature that detects all three mutations. Moreover, it is plain on the face of the Mensink *et al.* publication that only one primer pair and one probe is disclosed which, because of their position in the BCR and ABL genes, could not differentiate each of the e1a2, b2a2 and b3a2 BCR-ABL translocations. Likewise, it is equally plain on the face of the Eder *et al.* publication that only two primer pairs and two probes are disclosed which, because of the size of the amplicons generated by these primers, could not differentiate each of the e1a2, b2a2 and b3a2 BCR-ABL translocations. Eder *et al.*, page 1384, right column, final paragraph. The assertion that such methods could possibly distinguish each of the e1a2, b2a2 and b3a2 BCR-ABL translocations is scientifically unsupportable in addition to being a prohibited comparison under the law.

Because no *prima facie* case of obviousness has been established, or, in the alternative, any *prima facie* case of obviousness that may have been established has been rebutted, Applicants respectfully request that the rejection under 35 U.S.C. §103 (a) be withdrawn or reversed.

CONCLUSION

In view of the foregoing remarks, Applicants respectfully submit that the pending claims are in condition for allowance. An early notice to that effect is earnestly solicited. Should any matters remain outstanding, the Examiner is encouraged to contact the undersigned at the address and telephone number listed below so that they may be resolved without the need for additional action and response thereto.

Respectfully submitted,

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